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Neuron 49, 843–852, February 16, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.01.034

The Dynamics of Hippocampal Activation during Encoding of Overlapping Sequences

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Summary

Sequence disambiguation, the process by which overlapping sequences are kept separate, has been proposed to underlie a wide range of memory capacities supported by the hippocampus, including episodic memory and spatial navigation. We used functional magnetic resonance imaging (fMRI) to explore the dynamic pattern of hippocampal activation during the encoding of sequences of faces. Activation in right posterior hippocampus, only during the encoding of overlapping sequences but not nonoverlapping sequences, was found to correlate robustly with a subject-specific behavioral index of sequence learning. Moreover, our data indicate that hippocampal activation in response to elements common to both sequences in the overlapping sequence pair, may be particularly important for accurate sequence encoding and retrieval. Together, these findings support the conclusion that the human hippocampus is involved in the earliest stage of sequence disambiguation, when memory representations are in the process of being created, and provide empirical support for contemporary computational models of hippocampal function.

Introduction

The hippocampus is widely accepted to play a crucial role in memory (Eichenbaum, 2004; Squire et al., 2004). However, the exact nature of its contribution remains unclear. At the heart of several current theories is the notion that the hippocampus is critically involved in bridging discontinuities across time (Eichenbaum, 2004; Eichenbaum et al., 1999; Rawlins, 1985; Wallenstein et al., 1998). These models, drawing on the anatomical characteristics of hippocampal circuitry, particularly within the CA3 region, emphasize the role of the hippocampus in representing behavioral episodes as sequences of events (Eichenbaum, 2004; Levy, 1996; Levy et al., 2005; Lisman, 1999; Treves, 2004; Wallenstein et al., 1998). As such, the hippocampus is proposed to support episodic memory, our ability to recollect past experiences and "mentally replay" them (Tulving, 2002), in part through its ability to represent the temporal order of events (Eichenbaum, 2004). Moreover, according to one influential account, the relational theory, the hippocampus mediates the linkage of overlapping episodes (or event sequences) through their common elements, thus creating relational frameworks in which both the common and unique features of experiences are represented (Cohen and Eichenbaum, 1993; Eichenbaum, 2004). Thus the hippocampus, through its ability to represent networks of overlapping episodes (or journeys), is viewed to support a wide range of memory capacities including episodic memory, spatial navigation, and flexible memory expression, e.g., performance on transitive inference tasks.

Empirical evidence is consistent with an important role for the rodent hippocampus in memory for sequences. Rats with hippocampal lesions exhibit impaired memory for both sequences of nonspatial stimuli (e.g., odors) (Fortin et al., 2002; Kesner et al., 2002) and sequences of spatial locations (Kesner and Novak, 1982). Further, the phenomenon of "phase precession," whereby the firing of a given place cell occurs at earlier and earlier phases of theta cycles as a rat moves along a well-known path, suggests that the hippocampus represents the sequential order of places in a route (O'Keefe and Recce, 1993; Skaggs et al., 1996). There is less direct evidence supporting a role for the human hippocampus in sequence learning. Some studies suggest a role for the hippocampus (Fletcher et al., 2005; Mitchell et al., 2004; Schendan et al., 2003), whereas others do not (Grafton et al., 1995; Hazeltine et al., 1997; Willingham et al., 2002). The vast majority of these studies explore sequence learning in the context of the serial reaction time task (SRTT). In this task, subjects press a button corresponding to the location of a simple visual target on the screen, with successive targets following a defined spatial sequence. Given the well-recognized role of the hippocampus in many aspects of spatial processing (Burgess et al., 2002), hippocampal involvement in the SRTT may reflect, at least in part, the inherently spatial nature of the task. Outside the domain of the SRTT, there is limited neuropsychological evidence that the hippocampus plays a role in memory for sequences. Amnesic patients with selective hippocampal damage have been shown to have exhibited impaired memory for sequences of faces (Holdstock et al., 2005) and words (Shimamura et al., 1990) presented on a one-trial-only basis and for sequences of spatial locations in a radial arm maze presented over repeated exposures (Hopkins et al., 2004).

Sequence disambiguation, the process by which overlapping sequences are kept separate, has been highlighted as representing a key aspect of the hippocampal contribution to memory (Eichenbaum, 2004; Eichenbaum et al., 1999; Levy, 1996; Sohal and Hasselmo, 1998). In an empirical test of Levy's formal model of sequence disambiguation (Levy, 1996), rodents with hippocampal damage were shown to have impaired memory for a pair of overlapping odor sequences that had previously been learned to criterion (Agster et al., 2002). Specifically, lesioned rats were impaired on the critical part of the sequence after the segment of overlap between the two sequences, where memory for previous parts of the sequence (or preceding context) is essential to successful task performance. Further

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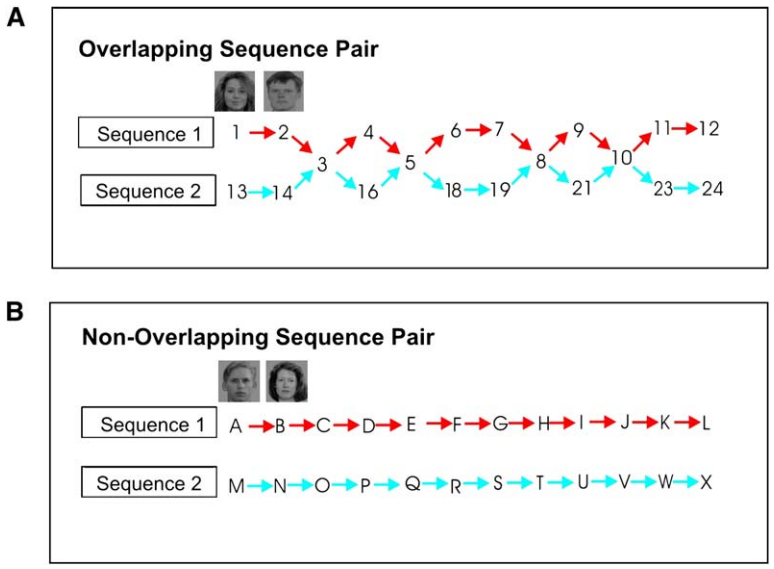

Figure 1. Schematic Representation of Overlapping and Nonoverlapping Pairs of Face Sequences

(A) Schematic representation (never seen by subjects) of OL sequence pair, consisting of sequence 1 and sequence 2. Each sequence consists of 12 faces, with four faces (numbered 3, 5, 8, and 10) common to both sequences. The first two faces of sequence 1 are shown for illustrative purposes. Red arrows are used to indicate the order of faces in sequence 1, and blue arrows for sequence 2. Subjects were required to learn one pair of sequences (OL or NOL) in each session.

(B) Schematic representation of NOL sequence pair. The two-face sequences constituting the NOL pair are entirely separate, with no faces common to both sequences. Letters are used instead of numbers to symbolize faces because faces comprising the NOL sequence were entirely different from those constituting the OL sequence. Red arrows are used to indicate the order of faces in sequence 1, and blue arrows for sequence 2.

evidence supporting the notion that the rodent hippocampus plays an important role in sequence disambiguation comes from single-cell recording studies during performance of spatial tasks, suggesting that the hippocampus maintains distinct representations for overlapping episodes (Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). In contrast, the putative role of the human hippocampus in sequence disambiguation has not previously been explored.

Here, we use functional neuroimaging to explore the role of the human hippocampus in sequence learning, outside the motor and spatial domains as previously studied with the SRTT. We selected faces as stimuli because they are naturalistic and not readily verbalizable. Moreover, faces can be considered to be nonspatial stimuli and therefore allow us to assess the role of the human hippocampus in sequence learning per se, independent of any contribution of spatial memory. A particular focus of this study was to test the hypothesis derived from computational models that sequence disambiguation involves the hippocampus in humans, as previously demonstrated by Agster et al. (2002), in rodents. In particular, we aimed to use fMRI to explore the dynamics of hippocampal involvement at the earliest stages of sequence disambiguation, during the encoding of overlapping sequences, at a time when memory representations are in the process of being created. Thus, we hoped to extend previous findings from rodent models of sequence disambiguation that have tended to focus on hippocampal involvement in sequence disambiguation after a considerable amount of training, when putative differential representations for overlapping episodes are likely to be well established (Agster et al., 2002; Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000).

In this fMRI study, we investigated the pattern of brain responses in 20 healthy right-handed subjects as they learned sequences of faces. Each subject took part in two contiguous scanning sessions and was required to learn one pair of face sequences during each session. There were two types of sequence pairs: either overlapping, i.e., both sequences in the pair share common faces (OL sequence pair), or nonoverlapping, i.e., entirely separate (NOL sequence pair) (Figures 1A and 1B). Thus, subjects were required to learn two pairs of face sequences during the entire experiment. Each of the two face sequences constituting a pair (either OL or NOL) was presented in separate 42 s "encoding blocks" and consisted of 12 faces, with each face presented for 3.5 s (Figure 2B). Subjects viewed each sequence five times over the course of the session (Figure 2A), with a retrieval test of sequence memory at the end of each encoding block providing a quantitative online measure of a subject's learning during each block. During each session, subjects also performed a control task matched to the sequence type (OL or NOL) in terms of stimulus composition (i.e., the number of different faces presented during each encoding block: see Experimental Procedures). During the control condition, faces were presented in a random order and subjects were instructed simply to pay attention to the faces themselves for a future memory test.

Thus, we set out to address three main issues. First, is the human hippocampus involved in learning sequences of nonspatial stimuli, outside the motor domain? Second, does the human hippocampus play a specific role in the learning of overlapping sequences? Third, is hippocampal activation at discrete points of the overlapping sequence of particular importance for the learning process? Specifically, current computational models (Eichenbaum, 2004; Levy, 1996; Wallenstein et al., 1998) propose that the hippocampus is integrally involved in coding of "local context," the representation of current events in relation to preceding and following events, implying that hippocampal activation at specific points in the overlapping sequence may be particularly important for accurate sequence encoding and retrieval.

Results

Behavioral Data

At the end of each sequence encoding block, there was a retrieval test made up of three "trials" each consisting of the presentation of four faces. Subjects were required

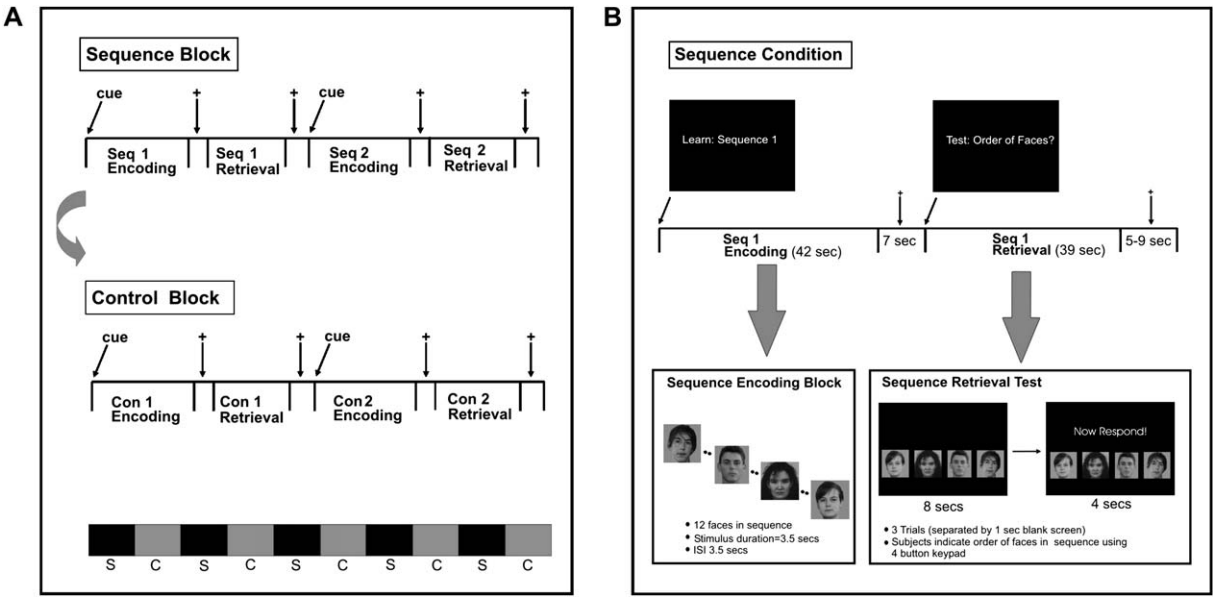

Figure 2. Experimental Design

(A) Timeline depicting sequence and control blocks. Each sequence block consisted of an encoding phase and a retrieval test phase for sequence 1 and then sequence 2 (always in that order), in addition to periods when a fixation cross was displayed. The organization of the control blocks was identical. Sequence (S) and control (C) blocks alternated with five blocks of each per session, as illustrated.

(B) Timeline depicting the encoding and retrieval test phases of sequence 1, i.e., one half of a sequence block. Cues indicating the onset of encoding and retrieval test phases are illustrated above the timeline. The composition of encoding and retrieval phases are illustrated below the timeline. See Experimental Procedures for design and instructions for the control tasks.

to indicate the relative order of the faces in the preceding sequence by using a keypad with four buttons (Figure 2B) (see Experimental Procedures). Thus, all 12 faces that composed the sequence were again viewed in the retrieval test. The purpose of this retrieval test was to provide a graded online measure of how much knowledge about the sequence subjects had acquired at several time points throughout the session. From this "retrieval score," we were able to deduce how much sequence knowledge had been acquired during each encoding block, termed the "learning rate" (see Experimental Procedures). As shown by Table 1, subjects were able to learn both OL and NOL pairs of face sequences over the course of five encoding blocks.

Table 1. Behavioral Measures

Parameter	OL Sequence	NOL Sequence	Control
Difficulty (1–10)	6.0 (1.6)	5.6 (1.6)	2.0 (3.7)
Overall sequence memory (/66)	64.6 (3.1)	63.9 (3.1)	n/a
Recognition memory (%)	99.2 (2.6)	97.9 (3.7)	96.9 (7.0)

Difficulty was rated by subjects in the postscan debriefing session on a scale of between 1 and 10 (10 = maximal difficulty). The score relating to overall sequence memory (maximum 66) relates to a test of subjects' memory for the entire two sequences, carried out at the end of each session outside the scanner (see Experimental Procedures). The recognition memory parameter relates to the percentage of faces previously seen during the experiment that subjects judged to be familiar, in a yes/no recognition test performed at the end of each session outside the scanner (see Experimental Procedures). All scores represent the average across the 20 participants. Standard deviations in parentheses.

Moreover, as predicted from initial pilot studies, subjects exhibited considerable variability in the rate at which they learned the pairs of face sequences (Figure 3), further motivating our use of a subject-specific behavioral index, the learning rate, in subsequent fMRI analyses.

At the end of each scanning session, memory for both sequences (constituting a pair) in their entirety was tested, outside the scanner (see Experimental Procedures). Performance on this task was excellent (Table 1) with no significant difference between OL sequence pair and NOL sequence pair ($t_{19} = 0.83$, $p = 0.42$). Subjects also underwent a recognition memory test at the end of each session, testing their memory for the faces themselves, from both the sequence and control conditions (see Experimental Procedures). Performance on this recognition memory test was also excellent for both control and sequence conditions (Table 1) with no significant differences between OL and NOL sequence types ($t_{19} = 1.14$, $p = 0.27$). Similarly, there was no significant difference between OL and NOL sequence pairs ($t_{19} = 0.87$, $p = 0.40$) in terms of subjective difficulty reported by subjects in a postscan debriefing session at the end of the fMRI experiment (Table 1). Unsurprisingly, subjects rated the sequence condition as significantly more difficult than the control condition ($t_{19} = 4.77$, $p < 0.001$).

Neuroimaging Data

Block-Related Analyses: Main Effects of Sequence Learning

We first contrasted all sequence encoding blocks (collapsed across sequence type) with control encoding

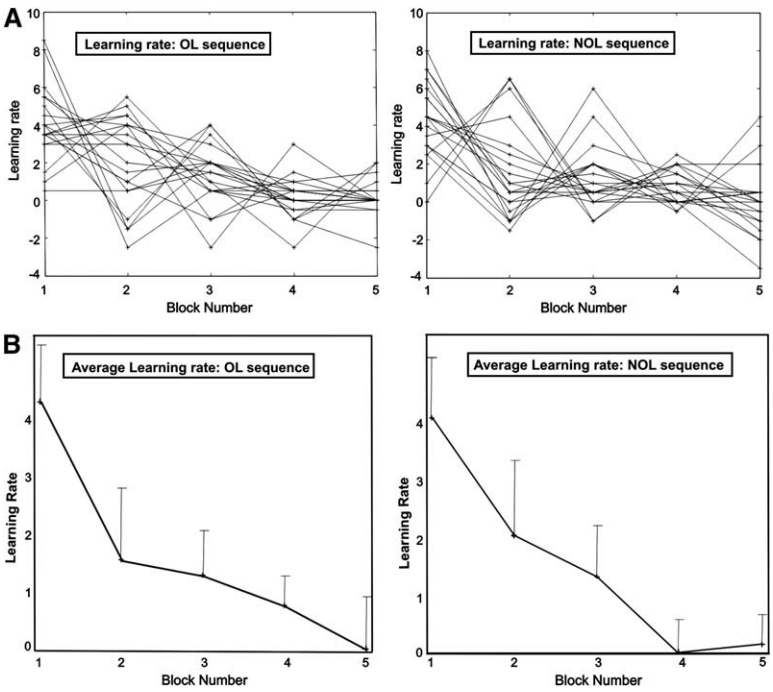

Figure 3. Learning Rate for OL and NOL Sequence Pairs

(A) Individual subject (n = 20) data during learning of the OL and NOL sequence pairs. An individual subject's learning rate is calculated from difference between successful scores on the sequence retrieval test (see Experimental Procedures). Thus, the learning rate for a given block reflects the amount of sequence knowledge acquired in the encoding phase from that block. Data is collapsed across sequence 1 and sequence 2 and plotted against block number (five sequence blocks in each session) for each subject.

(B) Group-averaged data of the 20 participants during learning of the OL and NOL sequence pairs. Data is collapsed across sequence 1 and 2 and plotted against block number. Bars reflect the standard deviation.

blocks in order to identify the general activation pattern associated with sequence learning across the entire experiment. There was significantly greater activation during sequence encoding blocks compared to the control condition within a well-defined network of brain regions (Figure 4 and Table 2) that has been previously implicated in mediating explicit sequence learning within the motor domain, in the context of the SRTT (Grafton et al., 1995; Hazeltine et al., 1997; Schendan et al., 2003; Willingham et al., 2002). However, in our study as in previous studies employing the SRTT, this network of activations may, at least in part, reflect the significantly greater attentional demands imposed by the sequence learning condition, which subjects rated as more difficult (Table 1), as compared to the control condition. We next contrasted the two sequence types (OL and NOL sequence pairs) with each other, collapsed across all encoding blocks across the session. No significant activations were observed in these comparisons, either when we compared the two sequence types directly, or through their respective control conditions in the form of an interaction contrast.

Block-Related Analyses: Learning-Related Change Collapsed across Sequence Type

Our primary interest in this experiment was to determine whether the hippocampus is involved in learning face sequences and specifically whether it has a particular role in learning sequences that overlap through common elements. We reasoned that if the hippocampus mediates sequence learning in this task, its activation during a given block should correlate with the amount of sequence knowledge acquired during that block. Therefore, we performed an analysis to look for brain regions whose activation pattern during the sequence encoding blocks exhibited a positive correlation with the learning rate (see Experimental Procedures). Of note, this approach has been used successfully in recent neuroimaging studies, in which hippocampal activation within a given encoding block was correlated with subject-specific behavioral indices of learning, for example the number of novel face-name associations learned within that block (Wolbers and Buchel, 2005; Zeineh et al., 2003).

We first considered the two sequence types (OL and NOL sequence pairs) together, i.e., collapsed across sequence type. In this analysis, activation in right posterior hippocampus (peak coordinate x, y, z) (21, −33, −12; z = 3.80) was found to show a significant positive correlation with learning rate (Figure 5). Thus, activation within a given encoding block in this region reflects the amount of sequence knowledge acquired, with greater activation during blocks where more is learned. To confirm that this finding reflects a specific correlate of the sequence learning process, we performed the following additional analyses (see Experimental Procedures): first, we included vectors coding for the learning rate as parametric regressors not only in the sequence condition but also in the control condition. Thus, we were able to identify brain regions showing a significantly greater correlation with the learning rate in the sequence condition as compared to a control condition matched in terms of stimulus composition. The pattern of activation in right hippocampus was very similar in this additional analysis to the original result, bolstering the conclusion that the correlation of activation in the right posterior hippocampus with learning rate is a specific correlate of sequence learning and not due to other factors such as nonspecific time effects or decreasing stimulus novelty. This conclusion receives further support from an analysis performed to look for a linear decrease over time in this region of the right hippocampus, which might be expected if activation in this region was modulated by changing stimulus novelty or was a reflection of nonspecific time effects. There was no significant linear

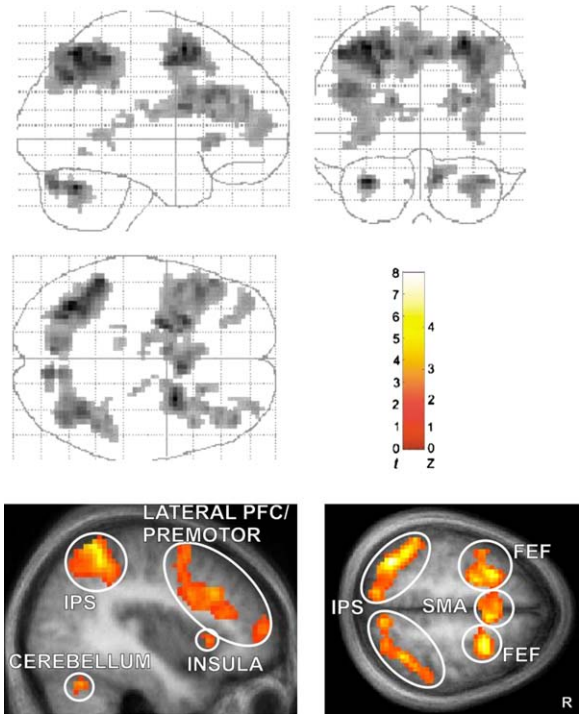

Figure 4. Main Effect of Sequence Learning

Brain areas significantly more active during the sequence condition compared to the control condition, collapsed across all encoding phases (see Results). Note, only the results of analysis of fMRI data from encoding phases is reported in this study. "Glass brain" figures are displayed above. Activations shown on the averaged structural MRI scan of the 20 participants (displayed below). IPS, intraparietal sulcus; SMA, supplementary motor area; FEF, frontal eye fields; PFC, prefrontal cortex; R, right side of the brain. The color bar indicates the t statistic associated with each voxel and the Z score equivalent. The threshold is set at $p < 0.001$ uncorrected.

decrease over time observed in this region during sequence or control conditions even at liberal statistical thresholds ($p < 0.01$ uncorrected). For the sequence condition, this was the case either when the analysis was collapsed across both sequence types, or considered separately for OL and NOL sequence types.

Table 2. Main Effect of Sequence Learning

Region	Laterality	x	y	z	Z Score
Intraparietal sulcus	L	−33	−63	54	6.07
	R	36	−57	45	5.06
Frontal eye fields	L	−24	12	54	5.38
	R	27	6	57	5.97
Cerebellum	L	−33	−63	−30	5.78
	R	39	−66	−30	4.72
Supplementary motor area	R	6	9	51	5.23
Lateral prefrontal cortex	L	−45	21	24	4.87
	R	39	45	24	4.45
Ventral premotor area	L	−45	9	30	4.59
Anterior insula	L	−36	21	−3	4.26
	R	33	27	0	4.37
Caudate	L	−12	−12	18	4.04
	R	15	0	21	4.70

Foci of activation for sequence versus control blocks, collapsed across the experiment. All values $p < 0.001$ uncorrected.

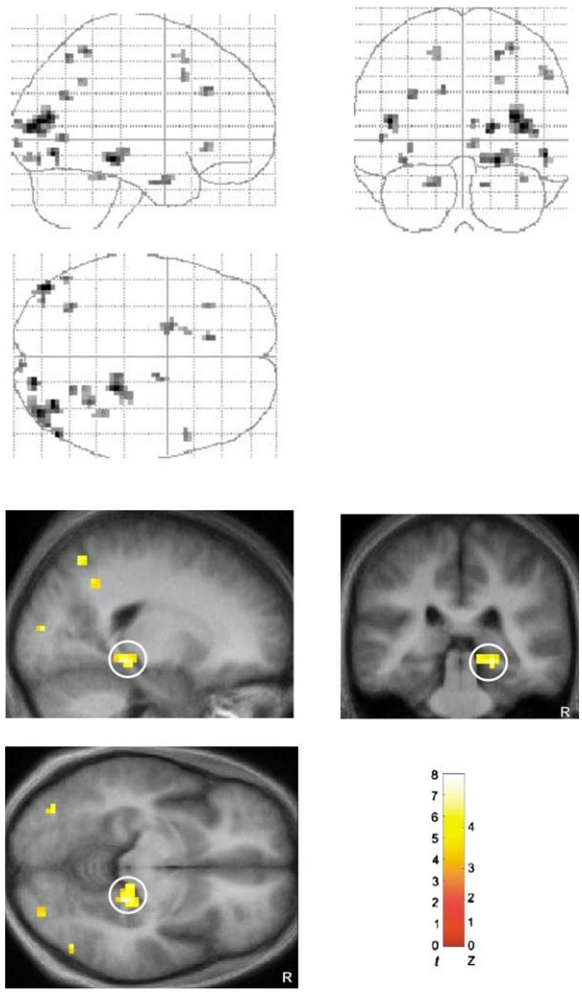

Figure 5. Learning-Related Effects Collapsed across Both Sequence Types

Brain areas whose activity during sequence encoding phases, when analysis is collapsed across both sequence types (OL and NOL), is significantly correlated in a positive manner with a subject-specific behavioral index of sequence learning, the learning rate. "Glass brain" figures are displayed above. Activations shown on the averaged structural MRI scan of the 20 participants (displayed below). R, right side of the brain. The color bar indicates the t statistic associated with each voxel and the Z score equivalent. Activation in right posterior hippocampus is circled in sagittal, coronal, and axial planes. Note threshold is set at $p < 0.005$ uncorrected for display purposes. Activation within R hippocampus is significant at $p < 0.001$ uncorrected.

Block-Related Analyses: Learning-Related Change Specific to Sequence Type

To test whether the hippocampus plays a greater role in learning overlapping sequences as compared to non-overlapping sequences, we next performed a region-of-interest (ROI) analysis in the right posterior hippocampus (see Experimental Procedures). This region was functionally defined from the group statistical map pertaining to the correlation with learning rate as described above, collapsed across both sequence types, and thresholded at $p < 0.005$ uncorrected. Thus, definition of this ROI is unbiased with respect to our contrast of interest: the direct comparison of OL and NOL

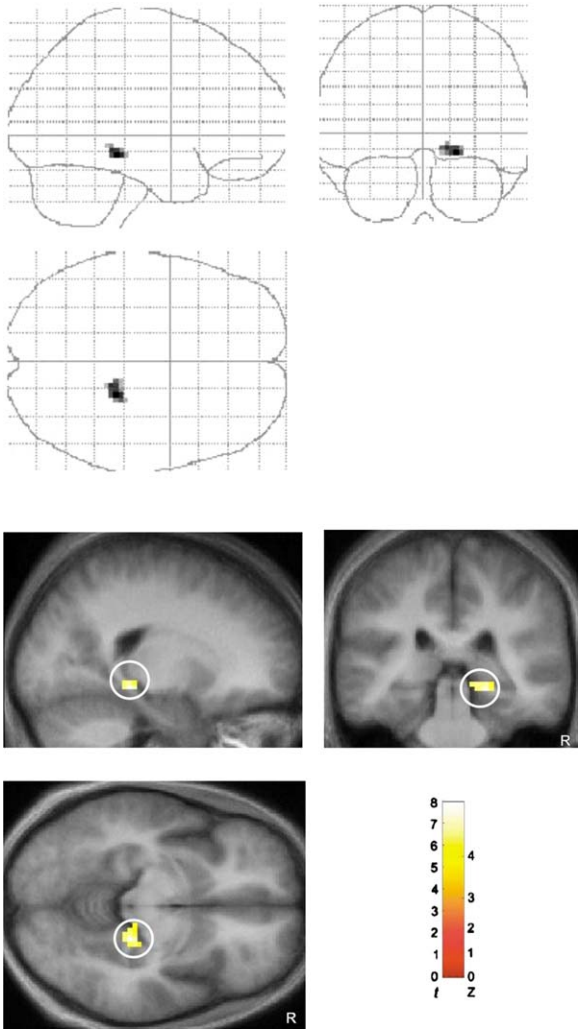

Figure 6. Learning-Related Effects Specific to OL Sequence Pair

Brain areas whose activity during OL sequence encoding phases is significantly correlated in a positive manner with a subject-specific index of sequence learning, the learning rate. "Glass brain" figures are displayed above. Activations shown on the averaged structural MRI scan of the 20 participants (displayed below). R, right side of the brain. The color bar indicates the t statistic associated with each voxel and the Z score equivalent. Activation in right posterior hippocampus is circled in sagittal, coronal, and axial planes. Threshold is set at $p < 0.001$ uncorrected. Activation within R hippocampus survives small volume correction using a bilateral hippocampal mask (see Experimental Procedures).

sequences. This analysis revealed that there was a significantly greater correlation of activation within the right hippocampal ROI with learning rate during OL sequence learning as compared to NOL sequence learning (t_{19} = 1.90, p = 0.037). These results suggest that this region in the right hippocampus plays a specific role in learning overlapping sequences.

Standard voxel-based analyses, considering the OL and NOL sequence pair separately, also support this conclusion. The same region in right posterior hippocampus (21, −33, −12; z = 4.15) shows a robust correlation with the learning rate in the OL sequence condition (Figure 6). In contrast, no significant correlation was observed in the NOL sequence pair even when liberal statistical thresholds were employed ($p < 0.01$ uncorrected).

Event-Related Analyses: Learning-Related Change Specific to Discrete Parts of the OL Sequence Pair

Thus, results from both voxel-based and ROI analyses suggest that the right posterior hippocampus plays an important role in sequence learning in our paradigm, predominantly when the sequences are overlapping. We next considered the possibility that the robust correlation of hippocampal activation with learning rate observed in the OL sequence condition might by driven by particular parts of the sequence, namely the faces common to both sequences ("common" faces) and those following immediately after ("after faces") (Figure 7). Accurate sequence encoding and retrieval of these key parts of the OL sequence has been predicted by computational models to be particularly reliant on the coding of local context; that is the representation of events (or faces) in relation to preceding and following events, a function proposed to be mediated by the hippocampus (Eichenbaum, 2004; Levy, 1996; Wallenstein et al., 1998).

Therefore, we carried out an event-related fMRI analysis in order to look for differential correlations of activation with learning rate in the right posterior hippocampus within distinct parts of the OL sequence pair. Thus, at the first level analysis, the OL sequence encoding blocks were subdivided into two separate regressors: one containing both "common" and "after" faces, and the other containing all "other" faces (see Experimental Procedures). The NOL sequence pair was subdivided in an analogous way according to ordinal positions of faces in the sequence in order to permit appropriate comparisons between the OL and NOL sequence pairs (see below and Experimental Procedures).

Results from this event-related analysis suggest that activation in the right posterior hippocampus in relation to the combination of "common" and "after" faces shows the most robust correlation with learning rate in the OL sequence. Voxel-based analyses demonstrate a robust correlation of activation with learning rate in the right hippocampus (21, −33, −12; z = 3.79) in relation to the combination of "common" and "after" faces (Figure 8) but not in relation to "other" faces even when a more liberal threshold ($p < 0.01$ uncorrected) was employed. Moreover, ROI analyses also support this conclusion: there was a significantly greater correlation of activation in the right hippocampal ROI with learning rate in relation to the combination of "common" and "after" faces as compared to "other" faces (t_{19} = 1.71, p = 0.05). It could be argued that this finding may in part reflect the greater number of faces within the "key" parts of the OL sequence as compared to the "other" parts. To consider this possibility, we compared correlations of activation with learning rate within the right hippocampal ROI, between analogous parts of the NOL sequence (see Experimental Procedures). Given that there was no significant difference in this comparison (t_{19} = −0.37, p = 0.64), we feel this is an unlikely explanation for the pattern of findings observed. Moreover, there was a significant difference in terms of learning rate correlation only when the OL "key" faces (t_{19} = 2.18, p = 0.02), but not the OL "other" faces (t_{19} = 0.33, p = 0.44), were directly contrasted with the analogous faces

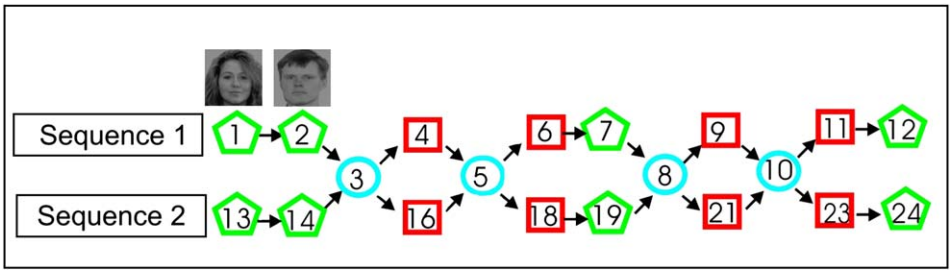

Figure 7. Schematic Representation of the Subdivision of the OL Sequence Pair

The OL sequence pair can be subdivided, in line with computational models of sequence disambiguation, into three different types of events, with each sequence composed of equal numbers of each type of event (i.e., four of each type): "Common" faces (circles), "After" faces (squares), and "Other" faces (pentagons). Event-related analyses designed to explore the correlation of hippocampal activation with learning rate in relation to each type of event was based on this classification (see the main text for details).

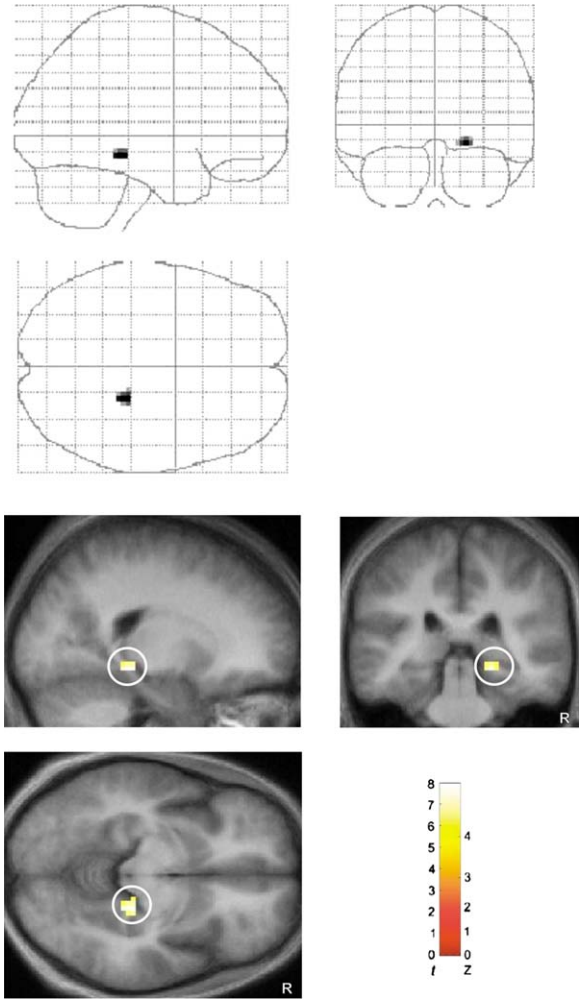

Figure 8. Learning-Related Effects within Specific Parts of the OL Sequence Pair: Event-Related Analysis

Brain areas whose activity in relation to the combination of "common" and "after" faces during OL sequence encoding phases is significantly correlated in a positive manner with the learning rate. "Glass brain" figures are displayed above. Activations shown on the averaged structural MRI scan of the 20 participants (displayed below). R, right side of the brain. The color bar indicates the t statistic associated with each voxel and the Z score equivalent. Activation in right posterior hippocampus is circled in sagittal, coronal, and axial planes. Threshold is set at $p < 0.001$ uncorrected.

in the NOL sequence. This further suggests that activation in the right hippocampus in relation to the combination of "common" and "after" faces in the OL sequence drives the robust correlation of hippocampal activation with learning rate observed in the OL sequence in previous block analyses.

These results suggest that the hippocampus plays a specific role in the learning of overlapping sequences, with activation in relation to the combination of "common" and "after" faces of particular importance. Given the design of the study, "common" and "after" faces are tightly coupled in the OL sequence, with "common" faces only presented immediately before "after" faces and vice versa. This tight coupling is a result of the structure of the OL sequence pair used in our paradigm, specifically the presence of overlaps consisting of only one face. The rationale for employing an OL sequence pair different from the one described in Levy's formal model (Levy, 1996) was to ensure that the OL sequence and NOL sequence pairs were matched not only in terms of overall length but also in terms of the overall number of sequential associations within each pair of sequences. Overlapping sequence pairs within which overlaps consist of more than one element contain less sequential associations than nonoverlapping sequence pairs of the same length. Further, we chose not to jitter the presentation of faces in order to preserve the paradigm's psychological validity.

Event-Related Analyses: Learning-Related Change Specific to Common Elements in OL Sequence Pair

The tight coupling of the "common" and "after" faces prevents the definitive assessment of their individual contributions to the robust correlation observed between activation in the right posterior hippocampus and the learning rate (see Experimental Procedures). However, in order to effect a partial decoupling of these two types of events, we performed two analyses: in the first analysis, we subdivided the OL sequence pair into two separate regressors at the first level, one containing just the "common" faces and the second containing both "after" and "other" faces. A highly significant correlation of activation in the right hippocampal ROI in relation to "common" faces was observed with the learning rate ($t_{19} = 2.88$, $p = 0.005$). In the second analysis, the OL sequence pair was again subdivided into two regressors, on this occasion one containing just the "after" faces and the second containing both "common" and

"other" faces. Here, no significant correlation of hippocampal activation with learning rate was observed in relation to "after" faces ($t_{19} = 0.84$, $p = 0.21$). Although the tight coupling of "common" and "after" faces in our paradigm prevents a definitive conclusion from being reached, this result suggests that activation in the hippocampus in response to "common" faces plays a key role in the learning of overlapping sequences.

"Common" faces, by definition, are viewed twice as often during the experiment, as compared to "after" or "other" faces. Therefore, we considered the possibility that the correlation of activation in the hippocampus in relation to "common" faces and learning rate might in part be due to changing stimulus novelty. In order to address this issue, we performed an analysis to look for a linear decrease over time of activation in relation to "common" faces in the sequence, which might be expected if activation in this region was modulated by changing stimulus novelty. No significant effects were observed in this region in this analysis even at liberal statistical thresholds ($p < 0.01$ uncorrected).

Discussion

In this study, we use fMRI to investigate the role of the hippocampus in learning sequences of naturalistic stimuli, outside the motor and spatial domains. We demonstrate that hippocampal activation during encoding of overlapping, but not nonoverlapping sequences, correlates robustly with a specific behavioral index of learning. Our findings further suggest that hippocampal activation at discrete points, where the two sequences comprising the OL sequence pair overlap, plays a pre-eminent role in the learning process. The present results, in providing evidence that the human hippocampus is intimately involved in the encoding of overlapping sequences, dovetail with contemporary theories of hippocampal function in which the hippocampus is proposed to mediate diverse aspects of memory, through its ability to represent both the common and unique elements among overlapping experiences (Cohen and Eichenbaum, 1993; Eichenbaum, 2004).

We observed a robust correlation of hippocampal activation with learning rate during the OL sequence condition, but not during the NOL sequence condition, suggesting that the hippocampus plays a specific role in the learning of overlapping sequences. Importantly, the marked difference between the OL sequence pair and NOL sequence pairs in terms of the correlation of hippocampal activation with learning rate cannot be explain by differences in difficulty or overall performance because the two sequence types were well matched across these parameters (Table 1). Rodent models of sequence disambiguation have provided insights into the role of the hippocampus at a stage when differential representations for overlapping sequences are likely to be well established, after a considerable amount of training (Agster et al., 2002; Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). Our study extends previous work in rodents by showing that the human hippocampus exhibits a dynamic pattern of activation that changes in parallel with learning, suggesting it plays an important role at an early stage of sequence disambiguation during the encoding of overlapping sequences. Thus, our results support the conclusion that the hippocampus not only maintains distinct representations for overlapping event sequences (or episodes), but is also actively involved in their creation.

We considered the possibility that differences in stimulus composition between OL and NOL sequence pairs might be contributing to the observed pattern of results: whereas there were 24 faces constituting the NOL sequence pair, there were only 20 faces in the OL sequence pair because of four faces being common to both sequences. We believe this to be unlikely for several reasons: firstly, the difference between the OL and NOL sequence pairs in terms of stimulus composition was small, consisting of only four out of 24 faces. Thus, any effect because of changes in stimulus novelty in the OL sequence condition would likely be present to almost the same extent in the NOL sequence condition. Second, activation in a brain region responding to changes in stimulus novelty would not be expected to show a robust correlation with a specific measure of sequence learning but instead be approximated by a linear decrease over time (Strange et al., 2005), which was not observed in our experiment in either the sequence or control conditions. Moreover, right posterior hippocampus has in several previous neuroimaging experiments been observed to show an increasing activation pattern as stimuli become more familiar, with more anterior regions in the hippocampal decreasing as stimuli become less novel (Strange et al., 1999, 2005).

Whereas a robust correlation was observed between hippocampal activation during encoding blocks in the OL sequence condition and learning rate, no significant correlation was apparent in the NOL sequence condition. Evidence suggesting that hippocampal lesions, in both rodents (Fortin et al., 2002; Kesner et al., 2002; Kesner and Novak, 1982) and humans (Holdstock et al., 2005) impairs memory for short sequences of items presented on a one-trial-only basis (odors or faces respectively), would seem to be at odds with our failure to find a significant correlation in the NOL sequence condition. However, it is important to note that our data does not preclude a role for the hippocampus in the learning of nonoverlapping sequences. Moreover, our paradigm in contrast to these studies, involved sequence learning over repeated exposures throughout the session. Thus, one possibility is that the learning of first-order conditional sequences, where one item leads unambiguously to the next, over repeated exposures as opposed to on a one-trial-only basis, may not be reliant on the hippocampus. This notion is in accordance with previous neuropsychological and neuroimaging research suggesting that the hippocampus is crucial for the learning of higher-order but not first-order sequences in the context of the SRTT (Curran, 1997; Schendan et al., 2003), through its ability to represent the higher-order associations among temporally distinct stimuli.

Results from event-related analyses whereby the OL sequence pair was fragmented into its constituent parts, show that hippocampal activation at specific points in the OL sequence may be particularly important for successful learning. Thus, activation in right posterior hippocampus in relation to the combination of "common" and "after" faces shows a robust correlation with learning rate that is significantly greater than the

correlation observed in the "other" faces. This finding is highly consistent with current computational models of hippocampal function because at these points in the OL sequence, hippocampal coding of local context is predicted to be particularly important for accurate sequence encoding and retrieval (Eichenbaum, 2004; Levy, 1996; Wallenstein et al., 1998). Contextual coding by hippocampal pyramidal cells is thought to be mediated by the formation, over repeated exposures, of "context units" that do not represent particular items in the sequence but instead support the "glueing" together of constituent parts of the sequence (Eichenbaum, 2004; Levy, 1996; Wallenstein et al., 1998).

Given the "common" and "after" faces were inextricably coupled in our paradigm, we were unable to directly compare the correlation of hippocampal activation in relation to these two types of events with the learning rate and thus assess their individual contributions to the observed findings. Nevertheless, the results of analyses where a partial decoupling was effected provide some evidence that activation in the right hippocampus in relation to "common," as opposed to "after," faces shows the most robust correlation with learning rate. This finding should be interpreted within the context of previous evidence from rodent models of sequence disambiguation (Agster et al., 2002; Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). Rodents with hippocampal lesions were selectively impaired on performing the "critical P5 choice," immediately after the point of overlap between the two sequences, where memory of preceding items in the sequence, or preceding context, is required to effect the correct choice (Agster et al., 2002). This result would seem to conflict with our finding of a more robust correlation of hippocampal activation in relation to "common" faces, as compared to "after" faces, with learning rate. However, as discussed previously, the deficit at choice P5 in Agster et al. (2002) reflects an impairment in the ability to disambiguate overlapping sequences at the retrieval stage, whereas in this study we assess the role of the hippocampus in the encoding of overlapping sequences. In our paradigm, "common" faces can be considered to be the counterpart of the central stem in a T maze, in experiments involving rodents performing a spatial-alternation task (Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). There is evidence to suggest that the rodent hippocampus is involved in the representation of differential codings for the common stem during left and right turn trials that serve to permit their disambiguation (Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Wood et al., 2000). Although the tight coupling of "common" and "after" faces in our design prevents a definitive conclusion from being reached, it is tempting to speculate that in our paradigm, hippocampal activation in response to "common" faces drives the separation of sequence representations that are overlapping, a process that is crucial for their subsequent disambiguation and, therefore, successful task performance. Thus, our findings also support the view that the hippocampus plays an important role in memory, in part through its ability to orthogonalize representations for overlapping input patterns, through a process of "pattern separation" (Marr, 1971; Norman and O'Reilly, 2003; Treves and Rolls, 1994).

One possible explanation for the observed difference between "common" and "after" faces in terms of correlation of hippocampal activation with learning rate is that "common" faces, by definition, were viewed by subjects twice as often as "after" faces. Although we cannot entirely exclude the possibility that differences in stimulus novelty contribute to the observed pattern of results, we believe this to be unlikely for similar reasons as previously discussed in relation to the comparison of overlapping and nonoverlapping sequence pairs. Thus, if activation in right posterior hippocampus in relation to "common" faces reflected the changing novelty of these items, then one would not predict that activity in this region would correlate robustly with a specific behavioral index of sequence learning, the learning rate. Instead, one would expect activation in this region in relation to "common" faces to be effectively modeled by a linear decrease over blocks, which was not observed in this experiment (Strange et al., 2005). An alternative explanation is that the correlation of hippocampal activation in response to "common" faces results primarily from the involvement of the hippocampus in sequence recall as opposed to sequence encoding per se, given the fact that it is not possible to fully dissociate encoding from retrieval processes in any learning paradigm. Although the hippocampus has been proposed to play an important role in sequence recall (Eichenbaum, 2004; Levy, 1996; Lisman, 1999), if this was the case, one would predict that hippocampal activation would correlate in a negative fashion with learning rate, as subjects acquired more knowledge about the OL sequence. Instead, the positive correlation of hippocampal activation in relation to "common" faces with learning rate observed in this study likely reflects its role in the process of encoding overlapping sequences.

Our results accord with previous neuroimaging studies suggesting that the posterior region of the hippocampus plays a role in sequence learning in the SRTT (Fletcher et al., 2005; Schendan et al., 2003). Indeed, the area in right posterior, medial hippocampus identified in our study is similar to that observed by Schendan et al. (2003) during explicit sequence learning, in contrast to a more anterior region they identified as engaged during implicit sequence learning. Why this particular region of the hippocampus is involved in sequence learning remains an open question. The posterior part of the hippocampus, particularly on the right in humans, has been implicated in spatial navigation in both rodents and humans, perhaps through its ability to store large-scale allocentric representations of the environment (Burgess et al., 2002). Indeed, the area of right posterior hippocampus identified in our study in the context of learning of overlapping sequences lies in close proximity to the region identified in previous neuroimaging studies as playing an important role in spatial navigation (Hartley et al., 2003; Kumaran and Maguire, 2005; Maguire et al., 2000). Moreover, there is evidence that use of an explicitly spatial strategy during task performance may, in some circumstances, result in engagement of the right posterior hippocampus (Maguire et al., 2003). Importantly, in our study, subjects reported that they did not use a spatial strategy during learning of the OL sequence pair when directly questioned in a postscan debriefing session. It is possible that our finding of a

significant correlation of hippocampal activity with learning rate reflects our use of faces as stimuli, given previous evidence suggesting that processes underlying the encoding of faces may preferentially occur within posterior regions of the hippocampus, predominantly on the right side, particularly under intentional learning conditions (Golby et al., 2001; Kelley et al., 1998; Small et al., 2001). Alternatively, the role of this region in learning overlapping sequences may be stimulus independent, perhaps mediated through its ability to encode higher order associations between stimuli discontinguous in time and/or space (Eichenbaum, 2004; Wallenstein et al., 1998). Thus, the creation of relational frameworks by the linkage of overlapping sequence representations through their shared elements might rely upon similar neural mechanisms as the representation of large-scale space.

In conclusion, our findings give support to current computational models that emphasize sequence disambiguation as a key aspect of the hippocampal contribution to memory. The present study extends previous work in rodents by demonstrating that the human hippocampus is involved in sequence disambiguation at the earliest stage, during the encoding of overlapping sequences when memory representations are in the process of being formed. In the future it will be important to determine the extent to which sequence disambiguation underlies the pervasive role of the human hippocampus in episodic memory and spatial navigation.

Experimental Procedures

Subjects

20 healthy, right-handed, native English speakers, who were currently undertaking or had recently completed a university degree, participated in this experiment (age range 21–30, average age 24.8, SD 2.7; ten female). All subjects gave informed written consent in accordance with the local research ethics committee.

Stimuli

A total of 140 grayscale front-facing photographs of unfamiliar male and female faces were used in this study. Images were obtained from the Stirling database (http://pics.psych.stir.ac.uk/) and cropped to remove external features present in the images, e.g., chairs on which subjects were seated. Examples of faces used in the experiment are shown in Figures 1 and 2.

All subjects were required to learn four sequences of faces in total (two in each session), consisting of 12 faces in each sequence. The OL sequence pair was composed of two sequences (sequence 1 and sequence 2) that overlapped through four faces common to both sequences (Figure 1). The NOL sequence pair was composed of two sequences that were entirely separate. Therefore, there were 20 different faces in total in the OL sequence pair and 24 different faces in the NOL sequence pair.

The control condition consisted of different faces from those that occurred in the sequence condition. However, the overall composition of stimuli in the control condition mirrored the composition of stimuli in the sequence condition. Thus, the control condition matched to the OL sequence pair consisted of two sets of 12 faces with four faces common to both sets. On the other hand, the control condition matched to the NOL sequence pair consisted of two sets of 12 faces, with no faces in common to both sets. As in the sequence condition, the four sets of faces in the control condition were equated for gender balance as well as attractiveness and distinctiveness, rated by a separate set of six subjects on a scale of 1 (low) to 7 (high) prior to the scanning experiment. The temporal order of presentation of the faces comprising each set in the control condition was different in each of the five blocks and pseudorandom in nature.

Tasks and Procedure

Scanning consisted of two main sessions lasting approximately 35 min each. During each of the two sessions, subjects were required to learn one of two sequence types: either the OL sequence pair or the NOL sequence pair. The order of exposure to sequence type was counterbalanced across subjects. During each session, subjects also performed a control task matched to the sequence task in terms of stimulus composition (see below).

Each scanning session consisted of five blocks of the sequence condition alternating with five blocks of the control condition (Figure 2A). Each of the blocks in the sequence condition lasted approximately 4 min and consisted of presentations of cues, encoding block, retrieval test, and fixation periods for first sequence 1 and then sequence 2 (always in that order) (Figure 2A). The control blocks were designed to mirror the sequence condition exactly in terms of overall format, presentation rate, and stimulus composition.

Subjects were familiarized with the task instructions outside the scanner prior to the main experiment with different faces to those employed in the main experiment. Subjects were told that in each session, they would be required to learn a pair of sequences, with each sequence consisting of 12 faces. They were instructed that they should do their best to learn the sequences as quickly as they felt able but that they would see each sequence five times over the course of the session. Further, in order to avoid surprise or confusion, subjects were informed that in one session, the pair of sequences to be learned would contain some faces that were common to both sequences. Subjects were not, however, explicitly informed that the sequences in this session would be "overlapping." Subjects were instructed that in the control condition, as in the sequence condition, they would see the same faces presented again and again for a total of five exposures over the course of the session. However, because the faces in the control condition would appear in a different (and random) order each time, there was no order to learn, and therefore they should not try to learn the order of faces in this condition. Instead, they were told simply to pay attention to the faces themselves, for a future memory test. Subjects were also instructed about how to perform the retrieval test (see below). In addition, subjects were told that their memory for the entire sequences would be tested at the end of each session and that they would also undergo a short recognition test for the faces themselves.

Each block began with a condition-specific cue displayed for 3.5 s: "Learn: Sequence 1" (sequence condition) or "Learn: Items 1" (control condition). Next, in a sequence encoding block lasting 42 s in total, 12 faces were presented one after another, each for a duration of 3.5 s (ISI 3.5 s), in the center of the screen on a black background (Figure 2B). These parameters were established following initial pilot studies prior to the scanning experiment. After this, a central fixation cross was displayed for 8 s. After this, a condition-specific cue was displayed for 3.5 s indicating that a retrieval test would shortly occur: "Test: order of faces?" (sequence condition) or "Test: recognize items?" (control condition). Each retrieval test consisted of three trials: in each trial, four faces were presented side by side in random positions (Figure 2B). Thus, over the course of three trials constituting the sequence retrieval test, all 12 faces that had been presented in the preceding encoding block were seen again. The array of four faces was then displayed for 8 s during which subjects either determined the relative order in which the faces had appeared in the preceding sequence (sequence condition) or determined which of the faces they had seen before (i.e., felt familiar to them) (control condition). The control retrieval test was organized in exactly the same way as the sequence retrieval test, although over the course of the session, three novel faces, never previously seen before by the subject, were included as foils in the array. Our reason for including only three foils in the control retrieval test was to ensure that, as far as possible, exposure was matched between sequence and control conditions, i.e., faces in sequence and control-encoding blocks, were seen an equivalent number of times by subjects. Further, the composition of arrays in the retrieval test (see below) was identical for all subjects. The sequence retrieval test was designed such that objective difficulty was approximately equal across the session. This was done by ensuring that, as far as possible, the degree to which faces in the array were separated from each other in the sequence was maintained constant over the course of five blocks. Subjects made their

response after a cue ("Now Respond!") that was displayed 8 s after the initial appearance of the array of four faces on the screen. Subjects had 4 s in which to respond, during which the four faces remained on the screen. Responses were made via an MRI-compatible four-button keypad. Subjects were instructed prior to scanning that each of the four keys corresponded to each of the four locations in which a face could be displayed on the screen during the retrieval test. In the sequence condition, they were instructed to depress the keys according to the order in which the faces had appeared in the preceding sequence. In the control condition, subjects were told to depress a key corresponding to a face that they recognized but not to press a key corresponding to a face that they judged unfamiliar. The next retrieval trial followed after a short blank screen (1 s) at the end of the response period of the previous retrieval trial. After the third retrieval trial, a central fixation cross was displayed for a variable period (5–9 s). At the end of this rest period, the second half of the block, identical to the first in format, began with the onset of the condition-specific cue: "Learn: Sequence 2" (sequence condition) or "Learn: Items 2" (control condition), signaling that the next encoding block was about to begin.

At the end of the first session, subjects were taken out of the scanner and their memory for the entire two sequences constituting the pair was tested. This was done separately for each sequence of the two sequences making up the pair, with memory for sequence one always tested first. For each sequence, 12 cards on which were printed the 12 faces comprising each sequence, were spread out on the table in a randomized array. For each sequence, subjects were required to order the 12 cards to best reflect the order in which the faces had been presented in the experiment. There was no fixed time limit for this task.

After completion of the overall sequence memory task, subjects underwent a computer-based yes/no recognition memory test for the faces themselves. Faces were presented at the center of the screen for 3 s each. This test comprised 88 faces in total, with an equal number of previously seen faces and foils. Subjects were required to respond by key press whether they had seen each face before.

After these two tests, subjects re-entered the scanner for the second session. The same testing procedure was carried out immediately after removal from the scanner at the end of the second scanning session. At the end of the scanning experiment, subjects participated in a debriefing session during which they were asked to rate the subjective difficulty of the tasks on a scale of 1 (very easy) to 10 (very difficult) and describe any strategies that they had used to aid learning in the experiment.

Behavioral Analyses

Subjects performed a retrieval test at the end of each of five encoding blocks in both the sequence and control conditions. In the case of the sequence condition, their performance on this task was used to create a subject-specific index of sequence learning, termed the "learning rate." This learning rate was derived from the "retrieval score" that indicated performance at the end of each block. In each of three trials that comprised the retrieval test at the end of each block, the order of faces indicated by subjects using the keypad was scored as follows: each face was awarded one point if in the correct position in the sequence relative to each other face in turn. Therefore the maximum score on each trial was six points (i.e., 3 + 2 + 1 = 6), with the maximum retrieval score over three trials therefore equating to 18 points. The learning rate was calculated by the difference between successful retrieval scores, reflecting the degree to which subject's performance improved with every block. Thus, the learning rate provides an online quantitative measure of the amount of knowledge about the sequence acquired during each encoding block. In order to calculate the amount of knowledge acquired in the first encoding block, the retrieval score at the end of this block was subtracted from a score indicating chance performance, i.e., nine points. Importantly, a learning rate was calculated for each of the two sequences comprising the pair that subjects learned during each session.

Performance on the task probing memory for the entire sequences, carried out at the end of the session (see above), was scored in an exactly analogous way to the retrieval test. Thus, each face in the subject's remembered sequence of faces was awarded one point if in the correct position in the sequence relative to each other face in turn. Thus the maximum score for this task, for each sequence, was 66 points (i.e., 11 + 10 + 9…+ 1 = 66). Performance on the yes/no recognition memory test was scored according to the proportion of faces previously seen in the experiment correctly judged by subjects to be familiar.

Neuroimaging Analyses

T2 weighted echo planar (EPI) images with BOLD (blood oxygen level dependent) contrast were acquired on a 1.5 tesla Siemens Sonata MRI scanner (Erlangen, Germany). We used standard scanning parameters to achieve whole brain coverage: 45 slices, 2 mm thickness (1 mm gap), TR 4.05 s. The first six volumes from each session were discarded to allow for T1 equilibration effects. Each session consisted of 504 volumes. A T1-weighted structural MRI scan was acquired for each subject after the two main scanning sessions. Images were analyzed in a standard manner with the statistical parametric mapping software SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Spatial preprocessing consisted of realignment, normalization to a standard EPI template in MNI space with a resampled voxel size of 3 × 3 × 3 mm and smoothing with a gaussian kernel with full width at half maximum of 8 mm. After preprocessing, statistical analysis was performed with the general linear model.

Block-Related Analyses

We targeted our analyses to detect brain regions whose activation pattern during sequence encoding blocks significantly correlated with a subject-specific index of learning, the learning rate. Thus, our interest was in the encoding blocks during which learning took place, in both sequence and control conditions. Hence, in the first level analysis, this 42 s period was modeled as a boxcar function and convolved with the canonical hemodynamic response function (HRF) to create regressors of interest. Subject-specific vectors coding for the learning rate, for each of the two sequences constituting the OL and NOL sequence pairs (i.e., four sequences in total), were then included as parametric modulators in the design matrix. These parametric regressors were also convolved with the HRF resulting in the height of the HRF for a given sequence encoding block being modulated as a function of the relevant learning rate for that particular block. Thus, these regressors model BOLD signal changes that covary with the learning rate for a given sequence. We also included vectors coding for the period of fixation between encoding block and retrieval test, as well as the retrieval test (including response periods) itself as regressors in the first level design matrix. Further, subject-specific movement parameters were included as regressors of no interest. A high-pass filter with a cutoff of 512 s was employed, appropriate for the cycle length in our paradigm. Subject-specific parameter estimates pertaining to each regressor (betas) were calculated for each voxel. Relevant contrasts of parameter estimates from all subjects were then entered into one-sample t tests (random-effects analysis).

Additional Block-Related Analyses

To confirm that our finding of a robust correlation between hippocampal activation and learning reflects a specific correlate of the sequence learning process, we performed two additional block-related analyses: in the first of these analyses, we included vectors coding for the learning rate as parametric regressors not only in the sequence condition but also in the control condition. Specifically, the parametric regressor coding for the learning rate for sequence 1 of the pair was included as a parametric modulator of the control 1 condition. The parametric modulator of the control 2 condition was derived from the learning rate for sequence 2. Apart from this alteration to the first-level design matrix, the analysis was conducted in an identical fashion to that previously described.

In the second of these analyses, parametric regressors coding for a linear time-dependent change were included in the design matrix as modulators of the sequence and control encoding blocks. In this model, no parametric regressors coding for the learning rate were included in the design matrix. Apart from this alteration to the first level design matrix, the analysis was conducted in an identical fashion to that previously described.

Event-Related Analyses

We carried out an event-related fMRI analysis in order to look for differential correlations of hippocampal activation with learning rate within distinct parts of the sequence. Thus, the OL sequence pair was fragmented into three types of faces (Figure 7): "common" faces, "after" faces, and "other" faces. The "common" and "after" faces were tightly coupled in our paradigm. Thus, the correlation with learning rate of hippocampal activation in relation to each type of face cannot be assessed with a model in which the OL sequence is subdivided into three separate regressors in the first-level design matrix. With this limitation in mind, we performed three separate analyses: in all three models the OL sequence was subdivided into two regressors in the first level design matrix, the difference between the three models determined by the composition of these two regressors. Regressors pertaining to the NOL sequence were always specified so as to mirror the subdivision of the OL sequence. In the first model, the first regressor contained both "common" and "after" faces and the second regressor contained "other faces." In the second model the first regressor consisted of "common" and the second regressor contained both "after" faces and "other faces." Finally, in the third model, the first regressor consisted of "after" faces and the second regressor contained both "common" and "other faces."

Subsequent analyses was identical for all three models: the presentation of each face in the sequence conditions was modeled as a boxcar function of 3.5 s duration and convolved with the canonical hemodynamic response function (HRF) to create regressors of interest. Subject-specific vectors coding for the learning rate, for each of the two sequences constituting the OL and NOL sequence pairs, were then included as parametric modulators in the design matrix. These parametric regressors were also convolved with the HRF. Thus, the height of the HRF for a given event was modulated as a function of the relevant learning rate for the block during which that event occurred. Thus, these regressors model BOLD signal changes in relation to specific parts of the sequence that covary with the learning rate. We also included vectors coding for the control conditions and retrieval test (including response periods) as regressors in the first level design matrix, as well as subject-specifc movement parameters. As in the block-related analyses, subject-specific parameter estimates pertaining to each regressor (betas) were calculated for each voxel. Relevant contrasts of parameter estimates from all subjects were then entered into one-sample t tests (random-effects analysis).

We also performed an additional event-related analysis to consider the possibility that the correlation of hippocampal activation with learning rate in relation to "common" faces might be due in part to the fact "common" faces were viewed twice as often as "after" and "other" faces. In this analysis, parametric regressors coding for a linear time-dependent change were included in the design matrix as modulators of the "common" faces. In this model, no parametric regressors coding for the learning rate were included in the design matrix. Apart from this alteration to the first level design matrix, the analysis was conducted in an identical fashion to that previously described.

Voxel-Based Analyses

We report results in a priori regions of interest (previously identified in neuroimaging studies of sequence learning in the context of the SRTT [Fletcher et al., 2005; Grafton et al., 1995; Hazeltine et al., 1997; Schendan et al., 2003; Willingham et al., 2002]) at $p < 0.001$ uncorrected for multiple comparisons, with an extent threshold of more than five contiguous voxels. In the case of the hippocampus, we report when activations survive small volume correction (SVC). This was performed with an anatomical mask drawn around the hippocampi bilaterally onto the average structural MR image for all participants. Activations in other regions are reported if they survive whole brain correction for multiple comparisons at $p < 0.05$.

ROI Analyses

To test whether the hippocampus plays a greater role in learning overlapping sequences compared to nonoverlapping sequences, we performed a region-of-interest (ROI) analysis in the right posterior hippocampus (with the MarsBaR SPM toolbox: http://marsbar.sourceforge.net/). This region was functionally defined from the group statistical map pertaining to the correlation of hippocampal activation with learning rate, collapsed across both sequence types, and thresholded at $p < 0.005$ uncorrected. Thus, definition of this ROI is unbiased with respect to our contrast of interest: the direct comparison of OL and NOL sequences. Using the MarsBaR SPM toolbox, we obtained parameter estimates for all voxels within this region, for the group as a whole. These parameter estimates were averaged across the ROI, and specific effects tested by one-sample t tests. It is important to note that these analyses treat data from an ROI as if it was from a single voxel and hence no correction for multiple comparisons is necessary.

Acknowledgments

This work was funded by a Wellcome Trust senior research fellowship in basic biomedical science to E.A.M. We thank H. Spiers and D. Hassabis for comments on earlier versions of the manuscript.

Received: October 21, 2005
Revised: November 29, 2005
Accepted: December 21, 2005
Published: February 15, 2006

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